

PDC isolation and culture

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An abbreviated version of this protocol was published in Science Translational Medicine in Sep 2020

PTEN status determines chemosensitivity to proteasome inhibition in cholangiocarcinoma

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Detailed protocol

PDC isolation and culture

Cells were isolated from PDX tumors as previously described with modifications. In brief,

1. PDX tumors were collected under sterile conditions and mechanically dissociated into small pieces (0.1-0.5 mm³) in a 60 mm dish. Add moderate RPMI 1640 medium when dissociating to keep cell viability.
2. Then samples were digested with collagenase I (10 mg/ml; Sigma) and trypsin (0.50%; Sigma) at a 37°C incubator. Mix the samples per 15 min and make sure the sufficient digestion. The optimal incubation period should be determined empirically.
3. The digested samples were filtered through a sterile 100-µm strainer. Transfer the filtered contents into a 15-ml centrifuge tube. Centrifuge at 1000 rpm, 3 min. Remove the supernatant.
4. Eliminate the red blood cells with a hypo-osmotic red blood cell lysis buffer (eBioscience) for 5 min. Centrifuge at 1000 rpm, 3 min. Remove the supernatant.
5. Resuspend the isolated PDCs with 2 ml RPMI 1640 medium. Transfer the mixture into a Matrigel-coated culture plates and culture cells in RPMI 1640 medium supplemented with 2 mM glutamine (Invitrogen), 20% FBS (Gibco), 1 µM dexamethasone (Sigma), 40 ng/ml human EGF (PeproTech), 20 ng/ml human FGF (PeproTech), 5 µg/ml human insulin (Sigma), 100 IU/ml penicillin (Gibco), and 100 µg/ml streptomycin (Gibco).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Dong, L., Tan, Y. and Wang, H. (2021). PDC isolation and culture. Bio-protocol Preprint. bio-protocol.org/prep987.
2. Jiang, T., Pan, Y., Wan, Z., Lin, Y., Zhu, B., Yuan, Z., Ma, Y., Shi, Y., Zeng, T., Dong, L., Tan, Y. and Wang, H. (2020). PTEN status determines chemosensitivity to proteasome inhibition in cholangiocarcinoma. Science Translational Medicine 12(562). DOI: [10.1126/scitranslmed.aay0152](https://doi.org/10.1126/scitranslmed.aay0152)

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